

Full Length Research Paper

Analgesic activity of *Ruta graveolens* L. (Rue) extracts

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The knowledge of pharmacological activity is mainly derived from the use that the population makes of plants. Rue (*Ruta graveolens* L.) is popularly used with a degree of mysticism due to its anti-inflammatory, healing, sedative and antispasmodic characteristics. In an attempt to confirm the well-known analgesic effects, the present study evaluated Rue extracts in animal models. Two organic extracts were produced and orally administered to mice, approximately 30 min before the tail immersion test in warm water ($55 \pm 0.5^\circ\text{C}$). The results reveal a small chronic analgesic effect and a high effect in acute assay (62.1% of pain inhibition) especially observed in the hexane extract, supporting the hypothesis of the use of this plant as having analgesic activity. However, the reports of the population about its analgesic activity should be caused also by its anti-inflammatory characteristics and the presence of compounds related in literature as analgesics. Further experiments should be done to better determine the dose-response analgesic effect of *R. graveolens* extracts.

Key words: *Ruta graveolens*, analgesic effect, tail immersion, rue.

INTRODUCTION

The *Ruta graveolens*, popularly known as Rue, from the Rutaceae family, aroused the interest of many researchers after reports on the traditional use of the plant by the population. The effects described were: diuretic, healing (Mendes et al., 2008), antiulcer (Raghav et al., 2006), antispasmodic, sedative and used in treatment of pain like toothache, pain in joints, headache and fever (Conway and Slocumb, 1979; San Miguel, 2003; Rodrigues et al., 2010; Khouri and El-Akawi, 2005; Raghav et al., 2006), and also antirheumatic. Studies have shown that Rue has potent anti-inflammatory activity due to the presence of the flavonoid rutin, which decreases nitric oxide production in macrophages inoculated without showing cytotoxicity (Raghav et al., 2006). Recent surveys show that the *R. graveolens* has abortifacient activity, (Roehsig et al., 2011);

antiandrogenic activity (Khouri and El-Akawi, 2005); potent antimicrobial activity (Mendes et al., 2008; Ivanova et al., 2005), antifungal activity (Meepagala et al., 2005; Ivanova et al., 2005), and antitumor activity (Hale et al., 2004; Preethi et al., 2006).

The Brazilian market is composed of a wide range of drugs, including anti-inflammatory and analgesic drugs from synthetic origin. However, herbal medicines have gained headway (Carvalho et al., 2008) after deployment policies for their use were implemented in National Health System by the Ministry of Health, provided that their production are ensured for safety, efficacy and quality.

With *R. graveolens* known as a common plant in Brazil with anti-inflammatory activity proven, this experimental study brings with it the possibility of developing new effective drugs in treating acute and chronic pain. Rue

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Table 1. Effect of oral administration of extracts of *Ruta graveolens* on response time of the animals in the tail immersion model.

Treatment (100 mg/kg)	Pain inhibition of extracts of <i>Ruta graveolens</i> in tail immersion test	
	Time of response (s)	Percentage of inhibition (%)
CR	1.3 ± 0.2	3.9
HR	3.6 ± 0.3*	62.1*
ASA	1.9 ± 0.5	17.6

These values represents pain inhibition in mean ± SEM and percentage (n= 4 animals). CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. *p ≤ 0.05 compared with the control group (ANOVA, Dunn's test).

will contribute to the development of new drugs and increases the possibility of therapeutic treatment of pain. Thus, this study aimed to evaluate the analgesic efficacy of *R. graveolens* through animal testing after oral administration of this plant extracts.

MATERIALS AND METHODS

Plant material and extraction

The sample of *R. graveolens* was taken from a planting backyard in the city of Cotia, São Paulo, in August, 2012. Two excisicates were deposited in the herbarium of the Mackenzie Presbyterian University identified with the number 1259 and 1260. The collected fresh plant (800 g) was submitted to an exhaustive extraction with the solvent methanol for 24 h at room temperature. After filtration, the residual was extracted twice in the same conditions. Subsequently, the ethanol extract was gathered and was subjected to a solvent partition in hexane and later in chloroform. The solvents were removed by evaporation at room temperature under a laminar flow. The resulting hydroalcoholic extract was discarded in this study due to the proliferation of a kind of yeast after drying. Finally, the dried extract was obtained: chloroform (CR; 730 mg) and hexane (HR; 255 mg). For the essays, the extracts were prepared at 2.5 mg/ml (100 mg/kg) in dimethylsulphoxide (DMSO; 2,0 ml/kg body weight).

Animals

Thirty-five *Mus musculus* species, lineage BALB/c mice were purchased from Federal University of São Paulo (female, 6 week-old) These animals were placed in the vivarium of the Mackenzie Presbyterian University under a 12 h light/dark cycle, controlled temperature (22 ± 2°C) and *ad libitum* feeding. On the day of treatment, food was withdrawn for about an hour before. All experimental animal procedures adopted for this study met the standards established by the Research and Ethics Committee of Mackenzie Presbyterian University and were previously submitted to that committee, with the number CEUA 046/05/2009.

Tail immersion test

This method was used for many author like Nogueira et al. (2005), Sharma et al. (2011) and Sathesh et al. (2010). The tail immersion test was performed by immersing, gently, the tail of the mice in warm water (55 ± 0.5°C). The animals were divided into five groups of four animals established as: saline group, negative control

(DMSO), positive control (acetylsalicylic acid), chloroform extract of *R. graveolens* and hexane extract of *R. graveolens*. For these extracts, an amount corresponding to the concentration of 100 mg/kg of their body weight was orally administered 30 min prior to the tail immersion test. The acetylsalicylic acid group received the same concentration (100 mg/kg) to it analgesic effect which could be comparable with the other groups. The cutoff time was set as 5 s to avoid damage to the animal as observed by Sathesh et al. (2010). Each animal had its time of tail removal timed and recorded with a stop watch. In this study, a single treatment was performed as acute essay, and a five day treatment as a subchronic essay (five-day treatment). Through the equation that relates the time of tail removal and the baseline value with the cutoff value, the pain inhibition (PI, percentage inhibition) could be calculated (Nogueira et al., 2005):

$$PI = \frac{\text{tail removal} - \text{baseline value}}{\text{cutoff value} - \text{baseline value}} \times 100$$

In this study, the baseline value was based on the time average of the saline group of each test day.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM) and were analyzed by analysis of variance (ANOVA), followed by Dunn's test and p < 0.05 was used as the significance level. Statistical analyzes were conducted using GraphPad Prism® software (version 6.01 GraphPad Software, Inc.). Dunn's test established significant correlation (p < 0.05) between the mean of all data presented.

RESULTS

Acute assay

This acute assay indicated an effect especially found in the HR extract, with a high value of 62.1% (Table 1). Despite the positive control (ASA) not being significant, it showed to be an analgesic agent with 17.6% of pain inhibition. In Figure 1 was clearly observed the effect of hexane extract of *R. graveolens* compared with the control group (DMSO) and the effect of the standard

Table 2. Effect of oral administration of extracts of *Ruta graveolens* on response time of the animals in the sub-chronic tail immersion model. Pain inhibition of extracts of *Ruta graveolens* in tail immersion test.

Days of treatment	Treatment (100 mg/kg)			
	DMSO	CR	HR	ASA
1	0.74 ± 0.08	1.16 ± 0.14	0.85 ± 0.08	1.54 ± 0.20 ^b
2	1.00 ± 0.07	1.36 ± 0.08	1.08 ± 0.12	1.96 ± 0.17 ^b
3	0.71 ± 0.05	0.97 ± 0.19	1.12 ± 0.21	1.17 ± 0.16 ^a
4	0.62 ± 0.04	1.08 ± 0.11 ^a	0.80 ± 0.07	0.73 ± 0.04
5	0.93 ± 0.11	0.82 ± 0.04	0.93 ± 0.02	1.33 ± 0.20

These values represents the time of removal (s) of tail in mean ± SEM (n= 4 animals). CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. ^ap ≤ 0.05; ^bp≤0.0001 compared with the control group (DMSO) of each day of treatment (ANOVA, Dunnett's test).

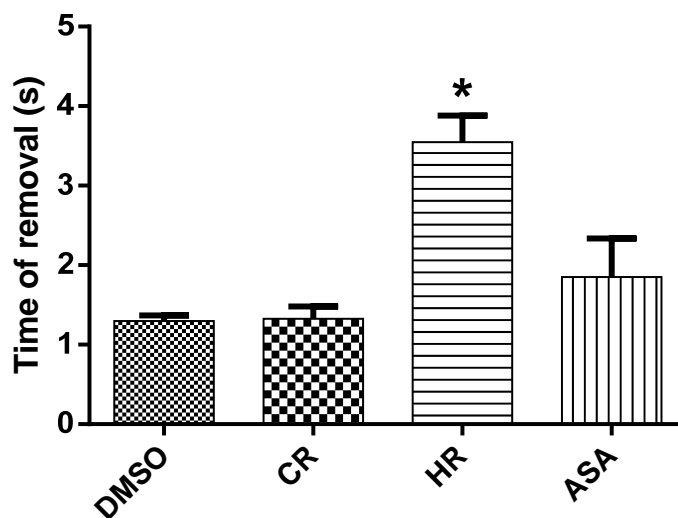


Figure 1. This Figure represent pain inhibition in mean ± SEM (n= 4 animals). DMSO= dimethylsulphoxide (2,0 ml/kg body weight); CR= chloroform extract of *Ruta graveolens* (100 mg/kg); HR= hexane extract of *Ruta graveolens* (100 mg/kg); ASA= acetylsalicylic acid (100 mg/kg). *p ≤ 0.05 compared with the DMSO group (ANOVA, Dunn's test).

(ASA).

second and third days of treatment, 17.8, 26.6 and 13.7% of pain in this model.

Sub-chronic assay

The sub-chronic assay was evaluated at the same model used in the acute. The animals receive daily (for 5 days) the extracts 30 min prior to the tail test. Although the acute assay demonstrated a high analgesic effect in the extracts of Rua, the results of sub-chronic assay showed lower results (Table 2). At the second day of treatment the CR extract showed maximum effect (12.1% of pain inhibition) and in third the HR extract (12.6% of pain inhibition). The standard group also demonstrated to be an effective analgesic drug inhibiting, respectively at first,

DISCUSSION

Our laboratory works with standard operating procedures (SOP), and the doses are standardized to 100 mg/kg when using plant extracts. Studies with other doses must occur to understand the dose-response effect. The solvent DMSO at 5% was used for its high capacity of dilution and low toxic effects (Jacob and Wood, 1967). Brown et al. (1963) observed that rats tolerate up to 10 daily doses of 10 ml/kg weight, and no effects was found. On this study was used only 2.0 ml/kg body weight.

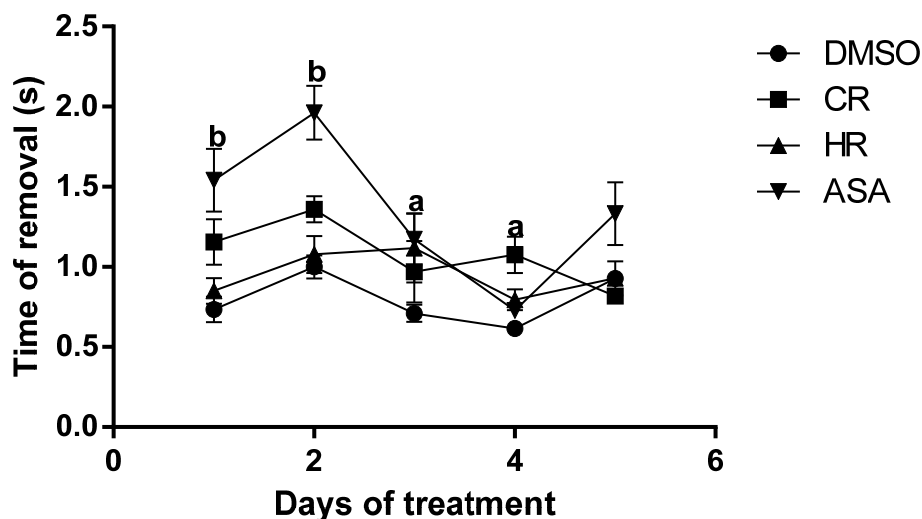


Figure 2. These values represents the time of removal (s) in mean \pm SEM (n= 4 animals) along the five days of treatment. CR= chloroform extract of *Ruta graveolens*; HR= hexane extract of *Ruta graveolens*; ASA= acetylsalicylic acid. ^ap \leq 0.05; ^bp \leq 0.0001 compared with the control group of each day of treatment (ANOVA, Dunnett's test).

Furthermore, Wilson et al. (1965) also demonstrated reduced side effects at 25% solution of DMSO, suggesting concentrations below 10%. Thus, Worthley and Schott (1969) showed no mortality in mice at dose of 10% and dose of 10.1 g/kg, this study used only 2.2 g/kg body weight. This procedure was first used as an experimental model seeking to understand the dynamics of the methodology. The Dunn's and Dunnett's test proved, with moderate evidence, the hypothesis that the extracts of *R. graveolens* increase the time of removal of the tail when compared to the negative control, at both essay (acute and sub-chronic), being responsible for the analgesia.

Although the effect was evidenced by the results analyzed, it was noticed as a sharp decline in the response of animals to the treatment with extracts of *R. graveolens*, a fact seen in the reduction of the time of removal of tail in the tested groups (Figure 2). This variation may be related to operant conditioning, where the animal realizes that removing the tail causes a relief from the pain induced, and removes it faster (Skinner, 1974; Desse and Hulse, 1975; Barbosa et al., 2007).

This study with organic extracts of *R. graveolens* confirmed the analgesic effect of Rue despite its low response in the sub-chronic model. The better extract analyzed was the hexane extract that showed to be effective on both essays. Kostova et al. (1999) and Sinshemko et al. (2000) describes the presence of a large amount of substances in the Rue as flavonoids (rutin), alkaloids (quinolines, furanocoumarins and furoquinolines), coumarins (furocoumarin and pyranocoumarin) and essential oils (2-nananone and 2-undecyl acetate). According to Atta and Alkofahi (1998), some of these components could be responsible for the

analgesic effect observed, among them the volatile oils, flavenoids and resins. Although it has analgesic effects, the Rue is known as an abortive plant, and many times is reported about its toxicity at high doses and in some cases even of death (De Freitas et al., 2005). The indiscriminate use by the population should be known by government health programs to teach the people about rational phytotherapy (Schultz et al., 1998) and to reduce the incidence of adverse effects related to this plant. Thus, this study support the results obtained by Atta and Alkofahi (1998) which concluded that Rue is an analgesic plant with dose-dependent response against thermal stimuli.

Conclusion

Despite the methodology only proving a small chronic analgesic effect of Rue, in the studied extracts, a high effect was observed in acute essay, supporting the hypothesis of the use of this plant as having analgesic activity. However, the authors believe that the reports of the population about its analgesic activity should be caused also by its anti-inflammatory characteristics extensively studied and it is the compounds related in literature as analgesics. In view of this, it is suggested that further research should be done to determine the best dose-response effect of extracts of *R. graveolens*.

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